Program/Abstract # 314
Neural development in two species of Engystomops (Anura: Leiuperidae)
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We analyzed the early neural development of *E. coloradorum* and *E. randi*. These frogs are endemic species from Ecuador that build foam nests on water surfaces and have a fast developmental rate. The small, white eggs of these frogs measure 1.3 and 1.1 mm in diameter, respectively. Neural morphology was analyzed in cross sections stained for cell nuclei. The neural plate and the neural tube were morphologically comparable to *Xenopus laevis* embryos. Embryos were immunostained against the neural cell adhesion molecule (NCAM) to detect the neural tube. The cranial neural crest (NC) was detected with an antibody against antigen 2G9. The NCAM immunostaining in whole mount strongly marks the neural tube and optic vesicles; in contrast the migrating NC cells were negative. The NC was detected in tail bud stage embryos that were immunostained against antigen 2G9. The cranial streams of NC cells were prominent and strongly positive. We identified the mandibular, hyoid, branchial anterior and branchial posterior streams of NC cells, and the neural tube. Rhombomeres 3 and 5 were negative for 2G9. Neural development was similar in the two species analyzed. The smaller embryos of *E. randi*, however, gave clearer immunostaining patterns. The neural pattern of development in these frogs is similar to that of other frog species. Large streams of cranial CN crest cells, however, are uncommon among frogs and have been detected before only in the marsupial frog *Gastrotheca riobambae*. This is the first description of neural development in *Engystomops*.

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Program/Abstract # 315
The contribution of Eph-Ephrin system to the maintenance of mesencephalon as a compartment
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A specific region in an embryo is often subdivided into subregions when embryogenesis proceeds. For example, the brain in a vertebrate embryo is subdivided into telencephalon, diencephalon, mesencephalon and rhombencephalon during the early development. In case of mesencephalon it has been shown that the precise position is determined by the mutual direct or indirect repression of transcription factors, Pax6-Engrailed/Pax2 and Otx-Gbx. Once the position of a subregion, or a compartment, is fixed, however, they must be further maintained; the cells in the compartment must be kept as one group, so that they do not intermingle with the cells in other compartments, at least for a certain time period. One way to keep cells as a group is to use homophilic cell adhesion molecules, e.g. cadherin. Another way is to employ a repulsive cell surface molecule system, e.g. Eph-Ephrin system. Eph-Ephrin system has been shown to be involved in some important biological processes including cell sorting, axon guidance or cell migration. Eph comprises a family of receptor tyrosine kinases and classified into two classes, EphA and EphB, based on the type of the ligands, which also classified into two classes, EphrinA and EphrinB, based on the molecular structure. Here, using overexpression experiments in the developing brain of chick embryos with in ovo electroporation we present the evidence that Eph-Ephrin system may contribute the maintenance of mesencephalon as a compartment. We also show that Eph-Ephrin system may contribute the overall morphology of the developing brain.

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Program/Abstract # 316
Zic2a and Hedgehog signaling in forebrain development
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The vertebrate forebrain controls many essential functions including memory and emotional regulation. ZIC2 and several Hedgehog (Hh) pathway members are essential for correct forebrain development in humans, but the mechanisms of their functions are not understood. To dissect these mechanisms, we have identified a novel early role for zebrafish Zic2a, a ZIC2 ortholog, during diencephalic development. We used knock-down and overexpression assays to show that zic2a is required and sufficient for transcription of foxb1.1, a marker of diencephalic precursors during late gastrula stages. Intact Hh signaling acting through GlI1 is required for the correct patterning of foxb1.1, but not for its transcription.
Foxb1 plays an essential role during development of the ventral diencephalon in mammals. Interestingly, depletion of Zic2a or Foxb1.1, or compromised Hh signaling, also results in a deficit of the prethalamus, a part of the ventral diencephalon, by 1 day post-fertilization. Zic and Gli proteins are hypothesized to physically interact in vivo. Although Zic2a and Gli1 both function during gastrulation to control development of the same forebrain precursors, we have preliminary evidence that suggests they function via different mechanisms. Live imaging analyses examining the dynamic morphological rearrangements in the anterior neural plate of Zic2a and Hh depleted embryos will be presented. These data provide novel insight into the roles of Zics, Foxb1 and Hh signaling in forebrain development. Our findings will help shed light on the genesis of holoprosencephaly (HPE), a prevalent forebrain defect that is caused by mutations in Hh signaling and ZIC2, and establish zebrafish as a model for HPE.

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Program/Abstract # 317
Lipoic acid synthetase is specifically required for forebrain formation in the mouse embryo
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Many human birth defects involve abnormal brain development. Studies of normal brain development in mouse embryos should elucidate both the origin and possible treatments of these human diseases. Here we describe the ENU-induced mouse mutant nearly headless whose most obvious phenotype is a severe truncation of the anterior brain. The initial specification of the forebrain is affected in nearly headless mutants. As development proceeds, the anterior truncation phenotype becomes more evident. As the formation of head region of mouse embryos depends on signals from the anterior visceral endoderm (AVE), the axial mesendoderm (AME) and the anterior definitive endoderm (ADE), we have examined marker gene expression of these embryonic structures. Our current data suggest that AVE and ADE are properly patterned in nearly headless mutants but there is a defect in axial mesendoderm specification. We have found that the nearly headless phenotype is caused by a partial loss of function mutation in Lipoic acid synthetase (Lias). Lias catalyzes the synthesis of lipoic acid, which is a crucial cofactor for several multienzyme complexes required for oxidative metabolism. This mutation causes a global defect in the cell cycle possibly by activating a cellular energy gauge, AMP-activated protein kinase (AMPK). We are currently investigating how a general defect in metabolism has a differential effect on forebrain patterning in the early embryo.

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Program/Abstract # 318
Analysing the role of Hoxa1 in mammalian hindbrain, inner ear and cardiovascular development
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Hoxa1 is one of the earliest and most anterior Hox genes expressed during embryogenesis. It plays an important role in regulating the development of the brainstem, inner ear and cranial ganglia in humans and mice. Patients with homozygous mutations in HOXA1 (Bosley–Salih–Alorainy Syndrome) exhibit severe defects in these structures. In addition, some patients display cardiovascular abnormalities. To analyse the function of Hoxa1 in the development of the brainstem, inner ear, cranial ganglia and the heart we performed genetics lineage analysis followed by conditional mutagenesis. Lineage analysis was carried out using a mouse line expressing Cre recombinase from the Hoxa1 locus. This technique allows us to genetically label all Hoxa1-expressing cells in the embryo and to follow their developmental fate. Our results demonstrate that Hoxa1-lineage is found in the caudal hindbrain with an anterior border in rhombomere 3. Additionally, we show that Hoxa1-expressing cells give rise to rhombomere 4-derived neural crest cells, which populate the second branchial arch and contribute to the VII/VIIIth ganglion complex. Hoxa1 lineage was also seen in a specific pattern in the otic epithelium and the outflow tract of the heart. In order to inactivate Hoxa1 function in each of these structures, we generated a Hoxa1-conditioned allele. This allele permits conditional inactivation of Hoxa1 function in one tissue at a time using Cre drivers specifically expressed in neural crest cell precursors, the otic placode and different hindbrain rhombomeres.

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Program/Abstract # 319
Functional analysis of novel genes differentially expressed genes in heart/hemangioblast precursor cells (H/HPC)
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Genetic evidence has implicated several genes as being critical for heart development. However, the inducers of these genes as well as their other targets and the pathways they constitute, remain largely unknown. The heart precursor cells are generated within bilateral fields in the lateral mesoderm, which consequently converge toward the midline to form a